

Stephen P.A. Fodor et al.
Application No.: 09/724,928
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PATENT

YG (A/G) (F/L) fits nicely with the outcome of a study in which a very large library of peptides on phage generated by recombinant DNA methods was screened for binding to antibody 3E7 (see Cwirla et al., Proc. Natl. Acad. Sci. USA, (1990) 87:6378, incorporated herein by reference). Additional binary syntheses based on leads from peptides on phage experiments show that YGAFMQ (SEQ ID NO:18), YGAFM (SEQ ID NO:19), and YGAFQ (SEQ ID NO:20) give stronger fluorescence signals than does YGGFM (SEQ ID NO:21), the immunogen used to obtain antibody 3E7.

Please insert the accompanying paper copy of the Substitute Sequence Listing, page numbers 1-6, at the end of the application.

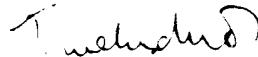
REMARKS

Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. The information contained in the computer readable disk of Application No. 09/442,027 was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

Attached hereto is a marked-up version of the changes made to the specification by the amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

The paragraph beginning on page 5, line 4 has been amended as follows:

Fig. 2 schematically illustrates one example of light-directed peptide synthesis (SEQ ID NOS:1, 2, and 22);

The paragraph beginning on page 5, line 6 has been amended as follows:

Fig. 3 is a three-dimensional representation of a portion of the checkerboard array of YGGFL (SEQ ID NO:1) and PGGFL(SEQ ID NO:2);

The paragraph beginning on page 16, line 25 has been amended as follows:

Fig. 2 is a flow chart illustrating another example of the invention. Carboxy-activated NVOC-leucine was allowed to react with an aminated substrate. The carboxy activated HOBT ester of leucine and other amino acids used in this synthesis was formed by mixing 0.25 mmol of the NVOC amino protected amino acid with 37 mg HOBT (1-hydroxybenzotriazole), 111 mg BOP (benzotriazolyl-n-oxy-tris (dimethylamino) – phosphoniumhexa-fluorophosphate) and 86 µl DIEA (diisopropylethylamine) in 2.5 ml DMF. The NVOC protecting group was removed by uniform illumination. Carboxy-activated NVOC-phenylalanine was coupled to the exposed amino groups for 2 hours at room temperature, and then washed with DMF and methylene chloride. Two unmasked cycles of photodeprotection and coupling with carboxy-activated NVOC-glycine were carried out. The surface was then illuminated through a chrome on glass 50 µm checkerboard pattern mask. Carboxy-activated $\text{Na}\alpha\text{-tBOC-O-tButyl-L-tyrosine}$ was then added. The entire surface was uniformly illuminated to photolyze the remaining NVOC groups. Finally, carboxy-activated NVOC-L-proline was added, the NVOC group was removed by illumination, and the t-BOC and t-butyl protecting groups were removed with TFA. After removal of the protecting groups, the surface consisted of a 50 µm checkerboard array of Tyr-Gly-Gly-Phe-Leu (YGGFL)(SEQ ID NO:1) and Pro-Gly-Gly-Phe-Leu (PGGFL)(SEQ ID NO:2).

The paragraph beginning on page 17, line 22 has been amended as follows:

In one example, the array of pentapeptides in the example illustrated in Fig. 2 was probed with a mouse monoclonal antibody directed against β -endorphin. This antibody (called 3E7) is known to bind YGGFL (SEQ ID NO:1) and YGGFM (SEQ ID NO:21) with nanomolar affinity and is discussed in Meo *et al.*, Proc. Natl. Acad. Sci. USA (1983) 80:4084, which is incorporated by reference herein for all purposes. This antibody requires the amino terminal tyrosine for high affinity binding. The array of peptides formed as described in Fig. 2 was incubated with a 2 μ g/ml mouse monoclonal antibody (3E7) known to recognize YGGFL (SEQ ID NO:1). 3E7 does not bind PGGFL (SEQ ID NO:2). A second incubation with fluoresceinated goat anti-mouse antibody labeled the regions that bound 3E7. The surface was scanned with an epi-fluorescence microscope. The results showed alternating bright and dark 50 μ m squares indicating that YGGFL (SEQ ID NO:1) and PGGFL (SEQ ID NO:2) were synthesized in geometric array determined by the mask. A high contrast(>12:1 intensity ratio) fluorescence checkerboard image shows that (a) YGGFL (SEQ ID NO:1) and PGGFL (SEQ ID NO:2) were synthesized in alternate 50 μ m squares, (b) YGGFL (SEQ ID NO:1) attached to the surface is accessible for binding to antibody 3E7, and (c) antibody 3E7 does not bind to PGGFL (SEQ ID NO:2).

The paragraph beginning on page 18, line 8, has been amended as follows:

A three-dimensional representation of the fluorescence intensity data in a portion of the checkerboard is shown in Fig. 3. This figure shows that the border between synthesis sites is sharp. The height of each spike in this display is linearly proportional to the integrated fluorescence intensity in a 2.5 μ m pixel. The transition between PGGFL (SEQ ID NO:2) and YGGFL (SEQ ID NO:1) occurs within two spikes (5 μ m). There is little variation in the fluorescence intensity of different YGGFL (SEQ ID NO:1) squares. The mean intensity of sixteen YGGFL (SEQ ID NO:1) synthesis sites was 2.03×10^5 counts and the standard deviation was 9.6×10^3 counts.

The paragraph beginning on page 32, line 19 has been amended as follows:

The identity of each peptide in the array could be determined from its x and y coordinate (each range from 0 to 31) and the map of Fig. 10. The chemical units at positions 2, 5, 6, 9, and 10 are specified by the y coordinate and those at [positions] positions 1, 3, 4, 7, 8 by the x coordinate. All but one of the peptides was shorter than 10 residues. For example, the peptide at x = 12 and y = 3 is YGAGF (SEQ ID NO:3) (positions 1, 6, 8, 9, and 10 are nulls). YGAFLS (SEQ ID NO:4), the brightest element of the array, is at x= 20 and y = 9.

The paragraph beginning on page 33, line 28 has been amended as follows:

The fifteen most highly [labelled] labeled peptides in the array obtained with the synthesis of 1,024 peptides described above, were YGAFLS (SEQ ID NO:4), YGAFS (SEQ ID NO:5), YGAFL (SEQ ID NO:6), YGGFLS (SEQ ID NO:7), YGAF (SEQ ID NO:8), YGALS (SEQ ID NO:9), YGGFS (SEQ ID NO:10), YGAL (SEQ ID NO:11), YGAFLF (SEQ ID NO:12), [YGAFL,] YGAFF ,(SEQ ID NO:13) YGGLS (SEQ ID NO:14), YGGFL (SEQ ID NO:15), YGAFSF (SEQ ID NO:16), YGAFLSF (SEQ ID NO:17). A striking feature is that all fifteen begin with YG, which agrees with previous work showing that an amino-terminal tyrosine is a key determinant of binding. Residue 3 of this set is either A or G, and residue 4 is either F or L. The exclusion of S and T from these positions is clear cut. The finding that the preferred sequence is YG (A/G) (F/L) fits nicely with the outcome of a study in which a very large library of peptides on phage generated by recombinant DNA methods was screened for binding to antibody 3E7 (see Cwirla et al., Proc. Natl. Acad. Sci. USA, (1990) 87:6378, incorporated herein by reference). Additional binary syntheses based on leads from peptides on phage experiments show that YGAFMQ (SEQ ID NO:18), YGAFM (SEQ ID NO:19), and YGAFQ (SEQ ID NO:20) give stronger fluorescence signals than does YGGFM (SEQ ID NO:21), the immunogen used to obtain antibody 3E7.